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USSR WORK ON MICROBIOLOGICAL METHODS OF PROSPECTING FOR PETROLEUM AND NATURAL GAS

[Comment: In reviewing the activity of the USSR microbiologist V. L. Omelyanskiy (1867-1928), A. A. Imshenetskiy, in an article entitled "In Memoriam, V. L. Omelyanski," Mikrobiologiya, Vol 22, No 4, 1953, says that microorganisms, being extremely sensitive reagents, may be used as indicators of the presence of combustible gases. The article on bacteria in the Bol'shaya Sovetskaya Entsiklopediya contains the following passage:

"About 30 years ago, V. L. Omelyanskiy referred to bacteria as chemical reagents and expressed the opinion that, in the future, bacteria will be used extensively for analytical purposes. Omelyanskiy's prediction has been strikingly confirmed. At present bacteria are used as exceedingly sensitive indicators which permit detection of traces of various vitamins, amino acids, and other compounds. The analytical procedures applied in this connection are based principally on the circumstance that some species of bacteria do not multiply in the absence of certain compounds, but do multiply when these compounds have been added to the nutrient medium. Some bacteria oxidize hydrocarbons (methane, propane, etc.). For that reason, detection of bacteria of this type indicates that petroleum or natural gas may be present in an underground stratum."

The following report summarizes two articles which have some bearing on Omelyanskiy's statement in regard to locating petroleum and natural gas deposits by the detection of bacteria associated with such deposits.

The first article, by L. I. Shturm, reviews the subject of petroleum microbiology, presenting data which have a bearing on petroleum formation and may be considered useful from the standpoint of potential or actual application in petroleum prospecting. The article is based, in part, on data collected by expeditions sent to the Second Baku oil fields in 1939-1940 by the Institute of Combustible Minerals, Academy of Sciences USSR.

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The second article, by L. K. Osnitskaya of the Institute of Microbiology, Academy of Sciences USSR, Moscow, deals with microflora at the bottom of the Caspian Sea between 47°E-55°E and 44°N-47°N. The article reviews her investigations and those of A. A. Maliyants and V. S. Butkevich and his collaborators. The article apparently does not have any bearing on the occurrence of petroleum, but the publication of Maliyants' investigations by the Azerbaydzhan Petroleum Institute indicates the institute's interest in marine microflora. This interest may indicate either the intention of locating offshore petroleum deposits or an attempt to collect data supporting the theory that petroleum hydrocarbons are formed as a result of bacterial action.

Numbers in parentheses refer to appended sources.7

Shturm's Article

The occurrence of microorganisms in strata containing petroleum deposits was established simultaneously in the USSR and the US in 1926. The assumption that the sulfate-reducing bacteria which occur in conjunction with petroleum deposits have the capacity of utilizing petroleum components would explain both the extensive occurrence of these bacteria in petroleum deposits, and the high level of sulfate-reduction processes that take place there. Utilization of petroleum hydrocarbons by sulfate-reducing bacteria has been established experimentally. One must assume that the activity of these bacteria is supposedly responsible for the absence of hydrogen, or the very low content of hydrogen, in petroleum gases. The fact that decomposition of sulfur-containing components of petroleum takes place under the influence of sulfate-reducing bacteria has been established.

Sulfate-reducing bacteria occur only in petroleum waters that contain hydrogen sulfide, which is the final stage of the reduction of sulfate ions.

On the basis of thermochemical relationships, it has been concluded that light paraffin hydrocarbons may be oxidized by bacteria to carbon dioxide with the utilization of the oxygen contained in water and in sulfates, while higher paraffins beginning with $C_{10}H_{22}$ or C_9H_{20} are converted mainly into naphthenes and polynaphthenes. It has been found that sulfate-reducing bacteria readily utilize aliphatic hydrocarbons of high molecular weight (e.g., tetradecane, eicosane, and hentriacontane), while decane is assimilated by these bacteria with difficulty. Apparently, hydrocarbons are assimilated selectively by sulfate-reducing bacteria. No work has been done which characterizes utilization of hydrocarbons by these bacteria on the basis of the hydrogen sulfide content.

There are indications that cyclic compounds are decomposed in connection with the bacterial reduction of sulfates. Some data indicate that the action of sulfate-reducing bacteria contributes to the liberation of petroleum from rock occurrences.

Sulfate-reducing bacteria are not invariably present wherever petroleum occurs. Some petroleum fields form exceptions in that respect.

Of great interest is another group of microorganisms, that of purpureal sulfur bacteria, discovered in Caucasian oil fields at the earliest stage of work on petroleum microbiology. These bacteria are also found in the water that occurs below the petroleum layer. They have the capability of oxidizing hydrogen sulfide to sulfuric acid and may therefore increase the sulfate content of the water. It has been demonstrated experimentally that they are capable of multiplying in the dark in media containing petroleum.

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In addition to the two groups of bacteria described above, microorganisms which reduce nitrates with the formation of nitrogen invariably occur in Tertiary petroleum deposits. These denitrifying microorganisms are able to use petroleum products as a source of carbon.

Anaerobic microorganisms which produce butyric acid fermentation, decomposition of proteins with the formation of gaseous products, and anaerobic fermentation of cellulose have also been found in petroleum deposits, but their relationship to the organic substances of petroleum deposits has not been clarified as yet.

In addition to anaerobic microorganisms, aerobic bacteria have also been discovered in USSR petroleum occurrences. An example is the thionic bacteria which oxidize thiosulfates, sulfides, and sulfur to sulfuric acid. Thionic bacteria occurring in the water below the petroleum layer are able to decompose the sulfur compounds of petroleum. Formation of oxygen in the layers where petroleum occurs can be explained by the action of radioactive compounds and by the catalytic effect exerted by some soluble salts, i.e., manganese chlorides.

The following possibilities exist for the formation of petroleum hydrocarbons by the action of bacteria: hydrogenation of unsaturated hydrocarbons, decarboxylation of saturated fatty acids, and reduction of carboxylic groups to methyl radicals. Contemporary microorganisms are incapable of achieving any of these conversions under anaerobic conditions, but extinct forms may have possessed the capability of forming petroleum hydrocarbons by the reactions mentioned. Conversion of fatty acids under rigidly anaerobic conditions can only lead to the formation of methane and carbon dioxide.

Some experimental data prove that higher hydrocarbons may form as a result of the action of microorganisms. It has been observed that gases formed in the decomposition of effluent sludge contain 0.4% ethane, 0.01% propane and butane, 0.07% pentane and hydrocarbons higher than pentane, 0.01% butadiene, 67.00% methane, 32.00% carbon dioxide, 0.03% carbon monoxide, 0.02% hydrogen, and 0.004% hydrogen sulfide. There are indications that sulfate-reducing bacteria synthesize hydrocarbons: when desulfovibrio /V. desulphureus/ microorganisms are cultivated in a medium that contains caproic acid, an oil which consists of the hydrocarbons C_{10} - C_{25} is formed.

The absence of hydrocarbons in present-day silt is explained by the activity of microorganisms which oxidize compounds of this type (i.e., hydrocarbons). Aerobic bacteria which are capable of oxidizing petroleum, refined petroleum products, and hydrocarbons in general occur extensively in marine silts. Their activity is inhibited by the presence of heavy metals, carbon monoxide, and hydrogen sulfide. It is also restricted by the low redox potential of the medium.

It is possible that accumulation of hydrocarbons as a result of bacterial activity could have occurred under specific conditions. Some investigators assume that the microflora of present-day petroleum deposits is genetically related to the microflora which existed in ancient bodies of water at the time when silt deposition took place there. The morphological resemblance between the sulfate-reducing bacteria of the Black Sea and the sulfate-reducing bacteria of the Groznyy petroleum strata supports this view.

The following conclusions can be drawn from data collected in the Second Baku area.

The petroleum crudes, the petroleum waters /water occurring below the petroleum deposit/, and petroleum-bearing rocks of the Second Baku contain living and biologically active microorganisms. The petroleum occurrences of the Second Baku are characterized by a microbiological biocoenosis composed of the following groups of bacteria: sulfate-reducing bacteria, nitrate-reducing bacteria, bacteria that oxidize hydrocarbons, bacteria that produce enzymatic decomposition of proteins, butyric acid fermentation bacteria, and bacteria producing ammonification. The microbiological biocoenosis of the deep layers of petroleum occurrences differs sharply from the microflora of the top layers of the earth and of the water of rivers, because it lacks such ubiquitous groups of microorganisms as aerobic and anaerobic bacteria which decompose cellulose, nitrification bacteria, and thionic bacteria. There is a correlation between the occurrence of sulfate-reducing microorganisms in petroleum waters and the presence of hydrogen sulfide, i.e., petroleum water that contains hydrogen sulfide also contains microorganisms which reduce sulfates. Apparently the presence of sulfate-reducing bacteria can be correlated with the presence of petroleum in certain geological structures. The large quantity of microorganisms present in the water occurring below the petroleum-bearing structures indicates that the petroleum may undergo various transformations under the action of the microorganisms.(1)

Osnitskaya's Article

A. A. Maliyants ("Microbiological Investigation of the Bottom of the Caspian Sea," Trudy Azerbaydzhanskogo neftyanogo issledovatel'skogo instituta (AzNII) /Works of the Azerbaydzhan Petroleum Investigation Institute/, Issue 18, Geological Division, page 1, 1933) has summarized the investigations carried out by her and by other scientists on the microbiology of the bottom of the middle part and the southern part of the Caspian Sea. In the opinion of Maliyants, the most widespread and intensive process which takes place at the bottom of the Caspian Sea is that of desulfurization. This process is brought about principally by Microspira aestuarii. Maliyants concludes from this that formation of hydrogen sulfide in the Caspian Sea is caused chiefly by the activity of desulfurizing bacteria. From all samples taken from the bottom of the sea, thionic acid bacteria (mainly Thiobacillus thioparus), purpureal sulfur bacteria, denitrifying bacteria (Th. denitrificans), nitrifying bacteria (Nitrosomonas), nitrogen-fixing bacteria (Azotobacter, Radiobacter), and other microorganisms could be isolated.

V. S. Butkevich and his collaborators ("On the Bacterial Population of the Caspian and Azov Seas, Mikrobiologiya, Vol 7, page 1005, 1938) established in the course of an investigation carried out in 1935 in the vicinity of the island Dolgiy that the surface of silt deposits is covered with a slime. He and other researchers expressed the opinion that this bacterial slime may further [illegible] as food to marine animals. He assumes that the bacteria contained in the slime convert decomposition products (carbon dioxide, methane, hydrogen and ammonia) into complex organic substances which may again enter into the nutrition cycle of living organisms.

The present investigation /by Osnitskaya/ was carried out with the aim of determining the extent of the film of bacterial slime at this time and also of establishing the quantitative relationship in this film of microorganisms which oxidize hydrogen sulfide, methane, and hydrogen, respectively.

The results of the investigation were as follows:

In the areas of the Caspian Sea investigated, the bacterial film described by Butkevich has not been found. Over the whole bottom, putrefaction bacteria, thionic acid bacteria, methane-oxidizing microorganisms, and hydrogen-oxidizing organisms were found to occur extensively at all depths of the sea and in all locations. Methane-oxidizing bacteria and hydrogen-oxidizing bacteria were represented in all types of bottom samples, the former in [illegible] of all samples, the latter in 70% of all samples. Thionic acid bacteria and putrefaction [illegible] were found in all bottom samples. Although the film described by Butkevich [illegible] been found, the surface layer of the bottom may be assumed to exert [illegible] tive function postulated by him, because hydrogen sulfide, methane, [illegible] are oxidized by the bacteria that occur in this layer.(2)

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/NOTE: In the area investigated, according to the data as arranged in Osnitskaya's diagrams, bacteria that oxidize methane are generally found together with bacteria that oxidize hydrogen. This evidently means that the substrate of the bacteria consists of putrefaction gases rather than petroleum gases -- hydrogen does not occur in petroleum gases or natural gas.

The reader is referred to the original article for diagrams illustrating the quantitative data mentioned in the article. Knowledge of the language is not necessary to interpret the diagrams. The percentages of bottom samples from which bacteria that oxidize methane were isolated are represented by rectangles with horizontal shading; the percentages of ground samples from which bacteria that oxidize hydrogen were isolated are represented by crosshatched rectangles. The corresponding values for putrefaction bacteria and thionic bacteria are represented by white and black rectangles, respectively. The data in the diagrams are arranged according to the composition of the bottom material (differences can be seen by comparing individual diagrams shown in each figure) and the depth of the water (differences can be seen by comparing Figure 2 with Figure 3). No breakdown according to geographic locations is given in the diagrams. On the map (Figure 1), a plus sign in square No 3 of the rectangular symbols indicates presence of methane-oxidizing bacteria, a minus sign their absence. The presence or absence of hydrogen-oxidizing bacteria is indicated similarly in square No 4./

SOURCES

1. L. D. Shturm, Data on the Microbiological Investigation of Petroleum Occurrences of Second Baku, Trudy Inst Nefti, Vol I, Issue 2, 1950, pp 97-119

2. L. K. Osnitskaya, Distribution of Putrefaction Bacteria, Thionic Acid Bacteria, Methane-Oxidizing Bacteria, and Hydrogen-Oxidizing Bacteria Over the Bottom of the Northern Part of the Caspian Sea, Mikrobiologiya, Vol XXII, No 4, 1953, pp 399-407

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